

USSN 09/308,207
Page 3

Amendment to the Claims

Claims 1-18. Canceled

19. (Currently amended): A recombinant microorganism capable of producing 1,3-propanediol from a carbon source said recombinant microorganism comprising

a) at least one introduced gene encoding a glycerol dehydratase from *Klebsiella* or *Citrobacter* or a diol dehydratase from *Klebsiella*, *Clostridium* or *Salmonella* activity;

~~b) at least one introduced gene encoding a glycerol-3-phosphatase isolated from *Saccharomyces*; and~~

e) and b) at least one introduced gene encoding protein X,

wherein the gene encoding protein X is i) isolated from a glycerol dehydratase gene cluster from an organism selected from the genera consisting of *Klebsiella* and *Citrobacter* or ii) isolated from a diol dehydratase gene cluster from an organism

selected from the genera consisting of *Klebsiella*, *Clostridium* and *Salmonella* and wherein protein X has no enzymatic activity, wherein production of 1,3-propanediol is

greater in the recombinant microorganism comprising protein X than in the recombinant microorganism lacking ~~absence of~~ said gene encoding protein X.

20. (Currently amended): The recombinant microorganism of Claim 19 further comprising ~~d)~~ c) at least one introduced gene encoding a protein selected from the group consisting of protein 1, protein 2 and protein 3, wherein said protein 1 has an amino acid sequence ~~of at least 95% similarity~~ corresponding to SEQ ID NO:60 or SEQ ID NO:61; said protein 2 has an amino acid sequence ~~of at least 95% similarity~~ corresponding to SEQ ID NO:62 or SEQ ID NO:63; and said protein 3 has an amino acid sequence ~~of at least 95% similarity~~ corresponding to SEQ ID NO:64 or SEQ ID NO:65

21. (Previously presented): The recombinant microorganism of Claim 19, wherein the microorganism is selected from the group of genera consisting of *Citrobacter*, *Enterobacter*, *Clostridium*, *Klebsiella*, *Aerobacter*, *Lactobacillus*, *Aspergillus*, *Saccharomyces*, *Schizosaccharomyces*, *Zygosaccharomyces*, *Pichia*, *Kluyveromyces*.

GC369-2A-USAM 09/04

USSN 09/308,207
Page 4

Candida, Hansenula, Debaryomyces, Mucor, Torulopsis, Methylobacter, Escherichia, Salmonella, Bacillus, Streptomyces and Pseudomonas.

Claims 22 - 25. Canceled

26. (Currently amended): The recombinant microorganism of Claim 19 wherein said dehydratase activity is heterologous to said microorganism.

27. (Currently amended): The recombinant microorganism of Claim 19 wherein said dehydratase activity is homologous to said microorganism.

28. (Currently amended): The recombinant microorganism of Claim 19 wherein the gene encoding protein X consists of nucleotides ~~0749-11572~~ a nucleic acid sequence of residues 9749 - 11572 of SEQ ID NO:19.

29. (Original): The recombinant microorganism of Claim 20 wherein protein 1 has the sequence as shown in SEQ ID NO: 60 or SEQ ID NO: 61.

30. (Previously presented): The recombinant microorganism of Claim 20 wherein protein 2 has the sequence as shown in SEQ ID NO: 62 or SEQ ID NO: 63.

31. (Previously presented): The recombinant microorganism of Claim 20 wherein protein 3 has the sequence as shown in SEQ ID: 64 or SEQ ID NO: 65.

Claims 32 -- 40. Canceled

41. (Previously presented): The recombinant microorganism of Claim 19, wherein the carbon substrate is selected from the group of monosaccharides, oligosaccharides, polysaccharides and one-carbon substrates.

42. (Previously presented): The recombinant microorganism of Claim 21, wherein the recombinant microorganism is an *E. coli*, a *Klebsiella* spp. or a *Saccharomyces* spp.

GC369-2A-USAM 09/04

USSN 09/308,207
Page 5

43. (Currently amended): The recombinant microorganism of Claim 19, wherein the further comprising a gene encoding the a glycerol-3-phosphatase is selected from the group consisting of a nucleic acid molecule encoding the amino acid sequence of SEQ ID NO:17, SEQ ID NO:33, and enzymatically active fragments thereof.

44. (Currently amended): The recombinant microorganism of Claim 19, wherein the gene encoding the dehydratase activity is a glycerol dehydratase of *Klebsiella pneumoniae*.

45. (Currently amended): The recombinant microorganism of Claim 19, wherein the gene encoding protein X is a nucleic acid molecule encoding the amino acid sequence of ~~SEQ ID NO: 66~~ SEQ ID NO: 67.

46. (Previously presented): The recombinant microorganism of Claim 19, wherein protein X is encoded by the ORF Z of the *Citrobacter* dha regulon.

47. (Currently amended): A recombinant *E. coli* capable of producing 1,3-propanediol from a carbon source said *E. coli* comprising

a) at least one introduced gene encoding a glycerol dehydratase from *Klebsiella* or *Citrobacter* or a diol dehydratase from *Klebsiella*, *Clostridium* or *Salmonella*, and

b) at least one introduced gene encoding protein X,

wherein

- a) protein X has no enzymatic activity,
- b) the gene encoding protein X is isolated from a glycerol dehydratase gene cluster from an organism selected from the genera consisting of *Klebsiella* and *Citrobacter*, or the gene encoding protein X is isolated from a diol dehydratase gene cluster from an organism selected from the genera consisting of *Klebsiella*, *Clostridium* and *Salmonella*,
- c) the carbon source is selected from the group of monosaccharides, oligosaccharides, polysaccharides and one-carbon substrates, and
- d) production of 1,3-propanediol is greater in the recombinant *E. coli* comprising protein X than in the recombinant *E. coli* lacking ~~absence of said~~ gene encoding protein X.

GC369-2A-USAM 09/04

USSN 09/308,207
Page 6

48. (Currently amended): The *E. coli* of Claim 47 further comprising at least one introduced gene encoding a protein selected from the group consisting of protein 1, protein 2 and protein 3, wherein said protein 1 has an amino acid sequence ~~of at least 95% similarity~~ corresponding to SEQ ID NO:60 or SEQ ID NO:61; said protein 2 has an amino acid sequence ~~of at least 95% similarity~~ corresponding to SEQ ID NO:62 or SEQ ID NO:63; and said protein 3 has an amino acid sequence ~~of at least 95% similarity~~ corresponding to SEQ ID NO:64 or SEQ ID NO:65.

49. (Currently amended): A recombinant microorganism capable of producing 1,3-propanediol from a carbon source said microorganism comprising

a) at least one introduced gene encoding a glycerol dehydratase from *Klebsiella* or *Citrobacter* or a diol dehydratase from *Klebsiella*, *Clostridium* or *Salmonella* ~~activity~~;
and

b) at least one introduced gene encoding protein X, wherein the gene encoding protein X

(1) consists of ~~nucleotides 9749-11572~~ a nucleic acid sequence of residues 9749-11572 of SEQ ID NO: 19;

(2) is an isolated nucleic acid molecule that hybridizes with (1) under the following hybridization conditions 0.1 x SSC, 0.1% SDS at 65°C, or

(3) is an isolated nucleic acid molecule that is completely complementary to (1) or (2),

and wherein production of 1,3-propanediol is greater in the recombinant microorganism comprising protein X than in the recombinant microorganism lacking ~~absence of~~ said gene encoding protein X.

50. (Currently amended): The microorganism of Claim 49 further comprising at least one introduced gene encoding a protein selected from the group consisting of protein 1, protein 2 and protein 3, wherein said protein 1 has an amino acid sequence ~~of at least 95% similarity~~ corresponding to SEQ ID NO:60 or SEQ ID NO:61; said protein 2 has an amino acid sequence ~~of at least 95% similarity~~ corresponding to SEQ ID NO:62 or SEQ ID NO:63; and said protein 3 has an amino acid sequence ~~of at least 95% similarity~~ corresponding to SEQ ID NO:64 or SEQ ID NO:65.

GC369-2A-USAM 09/04

USSN 09/308,207
Page 7

51. (Previously presented): The microorganism of Claim 49, wherein the recombinant microorganism is an *E. coli*, a *Klebsiella spp.* or a *Saccharomyces spp.*

GC369-2A-USAM 09/04